Show Me a Sign

New tools to detect cancer cell death inside the body would give doctors an early indication if cancer therapy is working.

Killing malignant cells – it's the number one goal of almost all forms of cancer therapy in use today. In the era of modern cancer therapy, researchers have developed innumerable ways to kill cancerous cells by triggering apoptosis, a genetically programmed form of cell suicide. Cancer researchers constantly search for new weaknesses in a cancer cell's armor and when they identify one, they screen thousands of chemical and biological agents to see if any can attack the newly found Achilles' heel. These molecules then enter the drug development pipeline, and if the science bears out, will some day become part of the chemotherapeutic armamentarium used to treat cancer.

At the same time, however, cancer cells have their own bag of tricks at the ready to survive these onslaughts. With unstable genomes, a hallmark of cancer, they have the uncanny ability to evolve their own genetic and biochemical defenses to avoid the lethal effects of chemotherapy and radiation therapy, just as microbes develop resistance to antibiotics. As a result, even the most toxic of cancer therapies can fail at their intended mission in some cancer cells. Months, or even years later, cancer springs anew.

In the laboratory, this give and take is apparent under the microscope – malignant cells either live or die. But in the human body, this battle has largely been enacted out of view, making it difficult to tell if a given therapy is working. As a result, clinicians and patients must often wait months for a sign that chemotherapy or radiation therapy is working, and the consequences of being in the dark can be dire.

That darkness may soon give way to light, as researchers have identified biochemical signatures of apoptosis and are now trying to develop methods for detecting those signatures. Initial clinical trials with some of these apoptosis detectors have shown promise, and now investigators are bringing the power of nanotechnology to bear on this effort in order to create powerful systems that would ultimately be capable of both delivering an anti-tumor drug and monitoring, in real time, if it is triggering cell death. The development of a nanotechnology-enabled apoptosis detector is one of the strategic areas of emphasis of the NCI Alliance for Nanotechnology in Cancer.

Though this work is still in its early stages, its potential is a harbinger of things to come. "Having a real-time apoptosis detector would represent a significant advance in cancer therapy, one that would let us alter treatment to reflect how each patient responds to a given set of drugs soon after being given those drugs," remarked James Olson, M.D., last year at a symposium on cancer nanotechnology. Olson, an oncologist at the Fred Hutchinson Cancer Research Center, is a member of a team, headed by University of Washington radiologist Raymond Sze, M.D., that is developing an apoptosis detector with funding from the NCI's Unconventional Innovations Program (UIP).

The development of a real-time monitor of apoptosis would have a positive effect throughout the cancer enterprise in large part because of the serious problems that result from not knowing if and when therapy starts to work. For the patient, receiving a therapy that is not working means unnecessary suffering, both from the tumor continuing to grow and any side effects that accompany the ineffective treatment. Receiving ineffective therapy for longer than needed also delays the start of second-line therapies that might work. Worse still, the failed treatment can trigger genetic defense mechanisms in tumor cells that can render ineffective these second-line therapies using other drugs. This phenomenon is known as cross-resistance.

The current months-long lag between the start of therapy and the appearance of obvious signs of initial success or failure also affects how new therapies undergo clinical testing. Because of the possibility of cross-resistance, regulatory agencies such as the U.S. Food and Drug Administration (FDA) are reluctant to allow testing of new cancer therapies on anyone but those patients who have exhausted all other therapeutic possibilities. Unfortunately, such patients are far less likely to respond to *any* therapy, making it far more difficult to prove the benefits of an experimental therapy. This difficulty is particularly true for the new generation of molecularly targeted therapies that aim to stop tumor growth early in its progression. An available real-time apoptosis monitor might enable such drugs to be tested at the initial diagnosis of cancer with less concern that prolonged therapy, should it fail to work, would put patients at risk by letting their cancers grow unchecked for longer than necessary. Instead, getting an early sign that such an early therapy is not working would allow patients to receive conventional therapy more quickly.

Apoptosis signatures

Every normal cell in the body has a finite lifetime, succumbing to the rigors of life either because it is injured or because it is triggered to commit suicide. Apoptosis is as natural – and as important – as is its complementary process, cell division. For example, nerve cells in the juvenile human nervous system undergo massive apoptosis as the brain matures and unneeded neurons are eliminated. Each month during menstruation, a woman's body sheds the inner lining of the uterus because of apoptosis. Immune system cells known as cytotoxic T cells help keep the body healthy by causing virus-infected cells to undergo apoptosis. And without apoptosis during fetal development, humans would have webbed hands and feet instead of distinct fingers and toes.

Whether a cell lives or undergoes apoptosis depends on a delicate balance. On the one hand, cells receive growth and survival signals, largely from the cells that surround it. On the other hand, cells receive a variety of negative signals that are mostly generated inside the cell. Negative signals can be increasing levels of oxidants within the cell (hence the popularity of dietary antioxidants), or an accumulation of proteins that are assembled incorrectly. Many anti-cancer drugs induce cell suicide by damaging a cell's DNA, which is a powerful apoptosis signal.[1] Conversely, a hallmark of a malignant cell is the ability to disable apoptosis.

Apoptosis itself is a complex series of cellular events whose end result is the cell breaking into smaller, membrane-enclosed packages that the body's scavenger cells – macrophages and dendritic cells – can engulf and digest. Early during this breakup, the cell's membrane folds inside out. This event exposes the molecule phosphatidyl serine, normally found on the internal side of the cell membrane, to the cell's external environment, providing a "come-and-get-me" signal to scavenger cells. This signal is "received" by the molecule annexin V which is found on the surface of scavenger cells.

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If scavenger cells can use annexin V to detect ongoing cell suicide, so might cancer researchers, and indeed, annexin V is one of the most promising apoptosis detectors. Initial studies have concentrated on linking annexin V to radioactive isotopes and detecting them with various imaging devices, such as a gamma camera. One version, using ^{99m}Tc, was in phase II trials to detect apoptosis following cancer therapy, but these trials were stopped in large part because of its short residence time in the body and the short half-life of ^{99m}Tc. These characteristics make it difficult to obtain useful images much beyond 6 hours after injection, a problem given that it is unclear when apoptosis should begin after therapy, a time that is likely to vary for each type of tumor or even for each patient. Nevertheless, trials in humans have successfully shown that imaging data obtained using this agent correlate with clinical outcomes in patients with advanced lung cancer and lymphoma.[2]

Given this promise, researchers are taking a number of approaches to solve the limitations of this initial work. For example, a group of investigators at the M.D. Anderson Cancer Center in Houston, led by Chun Li, Ph.D., are using ^{111}In-labeled annexin V linked to polyethylene glycol, which should have both a longer radiological half-life and a longer lifetime in blood. NCI-sponsored preclinical studies have shown that this formulation can also detect apoptosis in the body. These studies were performed in mice treated with paclitaxel.[3]

Sze and his colleagues at the University of Washington and the Fred Hutchinson Cancer Center, are attempting to use nanotechnology to solve these problems. His team is coating iron oxide nanoparticles with annexin V, an approach that will avoid the use of expensive radioactive isotopes. The magnetic iron oxide particles, which could circulate safely for much longer periods of time, should be readily visible using standard magnetic resonance imaging.

While annexin V is a promising candidate for apoptosis detection, a team at Sandia National Laboratories and the University of New Mexico, led by Sandia investigator Timothy Boyle, Ph.D., have created a synthetic apoptosis detector. Their candidate, a relatively small molecule that should be easy to manufacture, also binds to phosphatidyl serine, though it does so even better than annexin V. Though this research is in its infancy, Boyle and his colleagues have shown that this novel probe can detect apoptosis in laboratory-grown cells. Future development work will include attaching this molecule to various nanoparticles to determine if such constructs would make good *in vivo* imaging agents.

Fellow UIP grantee James Baker, M.D., and his colleagues at the University of Michigan's Center for Biologic Nanotechnology in Ann Arbor, are taking a completely different approach to apoptosis detection. His group is focusing on a protein-degrading enzyme, capsase-3, that is a central player in apoptosis and that apoptotic cells release into the circulation during their death throes. To detect capsase-3 activity in blood, Baker's group uses a protein-like molecule that functions as a substrate for this enzyme. When capsase-3 chews up this reagent, it produces a fragment that begins to fluoresce, and this fluorescence can be detected using a fiber optic probe inserted in a vein. This apoptosis-signaling molecule is attached to nanoparticulate dendrimers, spherical biocompatible polymers (see January 31, 2005 story, "Zipping Together Dendrimers with DNA" http://nano.cancer.gov/media_nanotech_news_2005-01-31.asp) that Baker's group has been using to target chemotherapy drugs and imaging agents to tumors. "Ultimately, we'd like to combine an imaging agent, drug, and apoptosis detector in the same dendrimer formulation," explains Baker, "so that we can detect a tumor, treat it, and then asses our treatment all at the same time." To date, the apoptosis detector has identified chemotherapy-triggered cell death in mice, demonstrating the promise of this approach.

Indeed, the potential of apoptosis detection to aid in cancer therapy and new drug development efforts has not gone unnoticed by the oncology community. In the many nanotechnology and cancer symposia sponsored over the past year by NCI's Office of Technology and Industrial Relations (OTIR), the need for such a detector was often high on the wish list of clinicians. "We received a clear message from the clinical community that using nanotechnology to develop a real-time monitor of apoptosis should be a top priority for any new nanotechnology effort," says Greg Downing, D.O., Ph.D., OTIR director. "We've certainly taken that into account in our new Alliance for Nanotechnology in Cancer, and we're hoping for a strong response now from the research community." <

— Joe Alper

References

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